bio-protocol Preprint

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Culture and treatment of BMDMs

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An abbreviated version of this protocol was published in Science Advances in Jan 2021

MBD2 serves as a viable target against pulmonary fibrosis by inhibiting macrophage M2 program

DOI: 10.1126/sciadv.abb6075

Detailed protocol

1. Cut the back legs above the hip joint in order to access the femur and tibia, leaving knee and ankle joints in place, and debride the muscle and tissue from by rubbing with gauze.
2. Fill a petri dish with 70% ethanol and dip the cleaned bones into it for 5–10 seconds to sterilize their exteriors before keeping them in a sterile tube on ice until all the bones are flushed.
3. In a laminar flow hood using sterile utensils, cut both ends of the bone with scissors as close to the joints as possible. Fill 1 ml syringe with ice-cold PBS, insert the syringe needle into the bone and flush out the bone marrow into a centrifuge tube on ice. Flush 2–3 times until the bones are completely white. Dissolve any clusters by pipetting.
4. Centrifuge 1 times in PBS at 300g for 5 minutes.
5. Lysed red blood cells for 5 minutes with 1ml Red blood cell lysis buffer. After neutralized with 2ml PBS, centrifuged 1 times in PBS at 300g for 5 minutes.
6. Resuspend the cell pellet in PBS and count viable cells using a hemocytometer and trypan blue.
7. Dilute the cells into 10 mL of RPMI 1640+10% fetal bovine serum (FBS)+penicillin/streptomycin+30ng/ml macrophage colony-stimulating factor (M-CSF) and plate in petri dishes at a density of 2 x 10^6 viable cells per plate.
8. Place plates in a 37°C incubator with 5% CO_2.
9. RPMI 1640+10% fetal bovine serum (FBS)+penicillin/streptomycin+30ng/ml macrophage colony-stimulating factor (M-CSF) was changed every 2 days
10. Stimulated the cells with IL-4 (10ng/ml) at the indicated time points at day 7.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Yi, W. (2021). Culture and treatment of BMDMs. Bio-protocol Preprint. bio-protocol.org/prep739.
2. Wang, Y., Zhang, L., Wu, G., Zhou, Q., Yue, H., Rao, L., Yuan, T., Mo, B., Wang, F., Chen, L., Sun, F., Song, J., Xiong, F., Zhang, S., Yu, Q., Yang, P., Xu, Y., Zhao, J., Zhang, H., Xiong, W. and Wang, C.(2021). MBD2 serves as a viable target against pulmonary fibrosis by inhibiting macrophage M2 program. Science Advances 7(1). DOI: 10.1126/sciadv.abb6075